Hematological and antioxidant effects of Cicer arietinum ethanolic extract on the male rats treated with Sorafenib

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Abstract
The present study was conducted to estimate the hematological and antioxidant effect of Cicer artinum ethanolic extract with Sorafenib in male rats. Twenty four adult male rats were divided into four groups each group contain seven rats;G1(control negative group received distilled water along of period of study), G2(control positive group received 200mg of Sorafenib only), G3(received Cicer arietinum extract at dose (315mg/kg) and G4 group treated with Sorafenib at dose (200mg/kg/B.W.) and Cicer arietinum extract (315mg/kg). The results showed that there were a significant decrease (P≤ 0.05) in blood parameters of animals in G2 group as compared with all other treated groups. While the blood values of G3 group revealed a significant increase (P≤ 0.05) as compared with all other treated except in PCV level of G3 group showed a a significant increase (P≤ 0.05) when compared with G1. In addition, the results of G4 group showed a significant increase (P≤ 0.05) in all blood parameters levels as compared with positive treated group. Interlukin-6, Glutathione peroxidase levels in G2 group revealed significant decrease (P≤ 0.05) as compared with all other treated groups while the Malondialdehyde level in positive control group revealed significant increase (P≤ 0.05) as compared with all other treated groups , as well as Interlukin-6, Glutathione peroxidase levels in Cicer artinum and sorafenib group revealed significant increase (P≤ 0.05) as compared with G1 and G2 groups, except Glutathione peroxidase of Cicer artinum and sorafenib group showed no significant difference (P≤ 0.05) as compared with G1 group. In conclusion, Cicer arietinum seeds ethanolic extract contain potential source of polyphenolis demonstrated antioxidant and supportive properties against toxicity of chemotherapy drug Sorfenib.
Keywords: Sorafenib, Cicer Arietinum, hematological and rat.

1-Introduction

Sorafenib (Nexavar) is an oral inhibitor of multikinase proteins approved in 2005 for treatment of metastatic renal cell and advanced hepatocellular carcinoma (1) It causes many metabolic side effects, including diarrhea, hypertension, hand-foot skin reaction, and fatigue. There are a number of problems associated with the use of drugs in chemotherapy. These included cytotoxicity, drug resistance and induction of new tumors (2). So, several studies gave their attention on using herbal medical plant to minimize the dose of chemotherapy drug and then reduce the side effect (3). Cicer arietinum L. is a legume belonging to the Fabaceae family. It was a cultivated crop grown in tropical, sub-topical, and temperate regions (4). In English, it is known as Bengal gram, chick pea, or garbanzo bean, while in Bangladesh it is known as boot dal or booter dal (5). The plant is cultivated widely in Bangladesh, because of its edible seeds, which are consumed in the boiled, fried, and the cooked form. Intake of chick peas has been recommended for humans suffering from type II hyperlipoproteinemia with altered lipid profiles (6). The phytochemical analysis of Cicer arietinum seeds revealed the presence of carbohydrates, proteins, amino acids, fixed oils, phytosterols, alkaloids, Polyphenolic compounds and tannins, glycosides, saponins, amino acids, iron, phosphate, sulfate, and chloride. Is Cicer arietrinum an aphrodisiac, estrogenic, antioxidant, anti-inflammatory, hepatoprotective, anticancer, and many other pharmacological effects (7).Cicer arietinum seeds ferritin appropriately provides an effective means of controlling the iron deficiency due to a high iron content with better absorption, so it is supposed to be explored a safe and efficient functional source for iron supplement (8). The present study designed to evaluate the anti-oxidant effect of Cicer arietinum extract on the male rats treated with Sorafenib.

2-Materials and Methods

Animals: This experiment has been done at the Department of Physiology, Biochemistry and Pharmacology, College of the Veterinary Medicine/University of Baghdad. Twenty four adult male rats 3 months age, weight 150-160g have been kept under the suitable environmental situation, temperature range (22-25) C◦. The rats were housed in plastic cages dimension 35x25x15 cm in an animal house about 2 weeks before the experiment for acclimatization. They were feed on standard pellets and tap water.

Ethanolic extraction: The extraction of Cicer arietinum seeds performed by the Soxhlet apparatus using 70% ethanol and a temperature of 50c°. The dried powder of Cicer arietinum seeds put into a

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thimble part and placed in the extraction unit till the clear and colorless solvent appeared in the extraction unit. Then, the extract was filtered and evaporated to dryness by using a rotary evaporator with 40 °C and 200 rotations per minute for 4 hours. Later on, a thick, semi-solid mass of deep-yellow color of crude extract appeared. All the dried extract was collected and kept in a freeze at - 20c° until use (9).

Experimental design: Twenty-eight adult male rats were divided into four groups equally and treated orally by stomach tube for a period thirty days as following:

The first group(G1): Seven adult male rats were intubated with distilled water only, as a negative control group.

The second group(G2): Seven adult male rats were treated with Sorafenib at dose (200mg/kg/B.W.) as positive control group.

The third group(G3): Seven adult male rats were treated with *Cicer arietinum* extract at dose (315mg/kg).

The fourth group (G4): Seven adult male rats were treated with Sorafenib at dose (200mg/kg/B.W.) and *Cicer arietinum* extract (315mg/kg).

Preparation of Stock Solutions, Concentrations, and Doses: Stock solutions of *Cicer arietinum* extract at doses 150, 200, 250, 300, and 350mg/kg were prepared to get the final concentrations of *Cicer arietinum* extract included 15, 20, 25, 300 and 350 mg/ml respectively. Each 0.2ml was given to 100gm body weight. Then, the doses were adjusted according to the body weight of each male rat. The recommended human dose of Sorafenib 2.85 mg/kg B.W. according to (10) B.W. was converted to animal dose by multiplying the human dose by factor 6.2 (11), to get the final concentration of stock solution 8.855mg/ ml, in addition, every 0.2 ml of the stock solution of Sorafenib was given orally to each 100g of rat body weight by stomach.

Determination of Complete Blood Count: Blood was collected from each animal in each different group. The blood is usually taken from the animal eye and heart (12). The sample is collected by drawing blood into a tube containing an anticoagulant (K2 EDTA) (13). The test method (automated) used for whole blood analysis (Complete Blood Count - CBC) using the Sysmex XN-350 Hematology Analyzers (14).

Determination of Serum Interleukin 6 (IL-6): This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). According to (15).

Serum Malondialdehyde (MDA): The concentration of MDA in serum was determined according to Buege and Aust method (16).

Serum glutathione peroxidase (GPX): It was performed according to (17).

Statistical analysis: Statistical analysis of data was performed on the basis of One way and Two-way analysis of Variance (ANOVA) using a significant level of ($p<0.05$). Specific groups differences were determined using Least Significant Differences (LSD) as described by (18).

3. Results and Discussion

The results in table 1 showed that there were a significant decrease (P≤ 0.05) in Hb, RBC, PCV, MCV and MCHC blood concentration of animals in Positive control group in means values (9.95 ± 1.60, 5.89 ± 0.894, 33.5 ± 2.30, 44.1 ± 1.51, 28.7 ± 1.69) as compared with all other treated groups. Whereas Hb, RBC, MCV and MCHC. While the Hb, PCV, RBC, MCV and MCHC of Cicer artinum treated group revealed a significant increase (P≤ 0.05) in means (15.1 ± 1.48, 9.11 ± 0.851, 43.1 ± 1.49, 49.7 ± 2.21, 34.0 ± 1.54) as compared with all other treated with no a significant difference (P≤ 0.05) as compared with negative control group except in PCV level of Cicer artinum treated group showed a a significant increase (P≤ 0.05) when compared with control group. In addition, the results of Cicer artinum and sorafenib treated group showed a significant increase (P≤ 0.05) in Hb, RBC, PCV, MCV and MCHC levels in mean values (13.7 ± 1.54, 7.10 ± 0.995, 37.8 ± 1.67, 47.2 ± 1.55, 31.2 ± 1.68) as compared with positive treated group with a significant decrease (P≤ 0.05) as compared with Cicer artinum treated group and Negative Control group.

Table (1): Hematological effects of Cicer arietinum against Sorafenib exposure in male rats.

Parameters	Negative Control group	Positive control group	*Cicer artinum* treated group	*Cicer artinum* and sorafenib group	LSD
Hb	14.3 ± 1.95 a	9.95 ± 1.60 c	15.1 ± 1.48 a	13.7 ± 1.54 b	0.5301
RBC	8.27 ± 1.00 a	5.89 ± 0.894 c	9.11 ± 0.851 a	7.10 ± 0.995 b	0. 9372
PCV	41.2 ± 3.24 b	33.5 ± 2.30 d	43.1 ± 1.49 a	37.8 ± 1.67 c	1.096
MCV	48.4 ± 0.989 a	44.1 ± 1.51 c	49.7 ± 2.21 a	47.2 ± 1.55 b	1.7936
MCHC	32.3 ± 1.68 b	28.7 ± 1.69 c	34.0 ± 1.54 a	31.2 ± 1.68 b	1.8247

*a, b, c, d: means in the same raw with different superscripts differ significantly at probability value (P≤0.05).
* Mean ± Standard Deviation.

Interlukin-6, Glutathione peroxidase and Malondialdehyde: The results of Interlukin-6, Glutathione peroxidase and Malondialdehyde of the present study were shown in table 2 the Interlukin-6, Glutathione peroxidase levels in positive control group revealed significant decrease (P≤ 0.05) in mean values (21.5 ± 0.848, 3.52 ± 1.06) as compared with all other treated groups while the Malondialdehyde level in positive control group revealed significant increase (P≤ 0.05) in mean values (8.44 ± 1.95) as compared with all other treated groups , as well as , Interlukin-6, Glutathione peroxidase levels of Cicer artinum treated group showed a significant increase (P≤ 0.05) in mean values (38.4 ± 1.03, 9.11 ± 1.49) as compared all

other treated group while the Malondialdehyde level in Cicer artinum treated group revealed significant decrease (P≤ 0.05) in mean values (4.34 ± 1.90) as compared with all treated groups with no significant difference (P≤ 0.05) as compared with negative control group. Interlukin-6, Glutathione peroxidase levels in Cicer artinum and sorafenib group revealed significant increase (P≤ 0.05) in mean values (31.7 ± 0.970, 6.82 ± 1.56) as compared with positive control group and negative control group, except Glutathione peroxidase of Cicer artinum and sorafenib group showed no significant difference (P≤ 0.05) as compared with negative control group. In addition, Malondialdehyde level in Cicer artinum and sorafenib group revealed significant decrease (P≤ 0.05) in mean values (6.28 ± 2.01) as compared with positive control group.as well as, there was significant increase (P≤ 0.05) in Malondialdehyde level of Cicer artinum and sorafenib group as compared Cicer artinum and negative control group.

Table (2): Effect of sorafenib, Cicer artinum and Cicer artinum with sorafenib on IL-6 (Interleukin-6), GPX (Glutathione peroxidase) and MDA (Malondialdehyde) of male rats for 30 days.

Parameters	Negative Control group	Positive control group	*Cicer artinum* treated group	*Cicer artinum* and sorafenib group	LSD
IL-6	29.1 ± 1.27 c	21.5 ± 0.848 d	38.4 ± 1.03 a	31.7 ± 0.970 b	1.1503
GPx	6.29 ± 1.71 b	3.52 ± 1.06 c	9.11 ± 1.49 a	6.82 ± 1.56 b	1.6325
MDA	5.09 ± 1.91 c	8.44 ± 1.95 a	4.34 ± 1.90 c	6.28 ± 2.01 b	1.1492

* a, b, c: means in the same raw with different superscripts differ significantly at probability value (P≤0.05).
* Mean ± Standard Deviation.

1. Discussion

In this study, the most deleterious hematological effect (anemia) appeared in animals of Positive control group which dosed with 200mg/kg.bw of Sorafenib, this finding strongly supported by (19), when explained the adverse effect of Sorafenib, he was observed anemia in 44% of Sorafenib -treated patients and 49% of placebo-treated patients , in addition, in the present study the depressions in all levels of blood pictures of sorafenib treated group may be attributed to the role of sorafenib in bone marrow depression lead to inhibiting hematopoietic stem cell production by bone the decrease the levels of RBCs, the same result reported by (20). In contrast the results of present study showed the protective effect of Cicer arietinum in combination group animals which dosed with 200mg/kg.bw Sorafenib and 315mg/kg. B.W of extract, this result can be attributed to active ingredient compounds of Cicer arietinum, Isoflavones are diphenolic secondary metabolites that may be lowered LDL-C oxidation, are secreted by different types of cells in response to a variety of stimuli such as tissue damage or infection and regulated the immune response and other biologic process (28). The results of current study revealed that the lowest concentration of IL-6 appeared Positive control group (control positive), rats that exposed to 200mg/kg.bw of sorafenib showed statistical reduction in the level of IL-6 concentration, these results agreed with (29) who reported that many protein kinase inhibitor (Sorafenib) inhibit IL-6 activity in preclinical models, with promising results both in cancer cell lines and animal models Also, Madleen et al., (28) agreed with our findings in another study when they demonstrated that acute Myeloid Leukemia patients after treated with sorafenib lead to decrease IL-6 and IL-10 and their levels. While an improvement in results of IL-6 may be attributed to polyphenols that displayed antioxidant activity, lead to the maintenance of the physical properties circulation and normality of blood (21). These findings were in agreements with (22) and (23) , furthermore, Cicer arietinum are rich sources of zinc, folate and protein lead to given a benefit effect in conditions like anemia and menstruation, skin and hair disease (24). In addition, seeds of Cicer arietinum contained ferritin which had a high iron content and higher absorption, it is expected to be investigated as a safe and effective functional source for iron supplementation (25). So, Cicer arietinum L. was investigated as a possible source of Fe fortification. The increase in Fe content and bioavailability in enriched chickpea products showed that they might be provided a considerable amount of the daily Fe requirement, this result agreed with (26). While, the results of increasing all blood pictures levels in group treated with Cicer artinum and sorafenib group may be attributed to potential effect of Cicer artinum compounds in lessening the adverse effect of sorafenib by decreasing free radicals and improving blood pictures (27). Normally, Cytokines

rutin and gallic acid were identified as two of the 26 individual polyphenol peaks that had significant antioxidant activity (30). Whereas the decrease in Gpx and increase in MDA of sorafenib treated group can be explained by the production of oxygen radicals, it was parallel by an augmented lipid peroxidative index as evidenced by the significant increase in malondealdehyde (MDA) detected in serum of rats intoxicated with sorafenib compared with control rats suggesting an increased production of oxygen free radicals in rats (31). Highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondedialdehyde, a lipid peroxidation product, where the accumulation of excess free radicals may be responsible for the increased lipid peroxidation (31).

Conclusion

Cicer arietinum seeds ethanolic extract contain potential source of polyphenolis demonstrated antioxidant and supportive properties against toxicity of chemotherapy drug Sorfenib.

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